Would R.D. Lawrence Have Been Interested in the Regulation of Insulin Secretion from Pancreatic β-Cells?

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Dr Peter Jones gave the 1997 R.D. Lawrence Lecture to the Medical and Scientific Section of the British Diabetic Association. This prestigious award, made to an outstanding young researcher, is named in honour of the man who, with H.G. Wells, founded the British Diabetic Association, and was given to Dr Jones in acknowledgment of his work in the field of islet cell physiology and pathophysiology. In this article, Dr Jones recalls his lecture and describes the principles of intracellular signalling in insulin secretion and the need for beta-cells to live together. © 1998 John Wiley & Sons, Ltd.

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R.D. Lawrence, Scientist

> I would point out to diabetics and their friends that they owe their lives to medical research, and it ought to be their duty and pleasure to support it in further progress.[1]

R.D. Lawrence is remembered in many ways: as an insulin-dependent diabetic person whose life was saved by insulin, who subsequently dedicated that life to understanding and treating the condition; as an outstanding clinical diabetologist whose long career stretched from the era of the discovery of insulin to the mid-1960s; and as a founder and advocate of the British Diabetic Association. He is perhaps less well remembered as a champion of medical research and a defender of the scientific method, but even a cursory reading of his published work reveals a rigorous scientist whose attention to experimental detail is a lesson to all of us who consider ourselves to be research scientists, whether clinical or basic.

For example, Lawrence had no doubt as to the value of constructing a hypothesis and putting it to impartial test by experiment.

> …the investigator must be careful to be fair and open-minded and guard himself against a bias in favour of the darling child of his own imagination. It is all too easy either to gloss over a few discordant results or to weigh the evidence in favour of one's own hypothesis.[2]

To Lawrence, control experiments were an integral aspect of any investigation…

> Therefore a controlled experiment should always be devised which removes, as far as possible, all the other varying conditions than the exact relation to be studied.[2]

…and he could be scathing about colleagues who did not apply the appropriate scientific methods to their studies.

> The error… has arisen from the clinicians concerned being unable to appreciate what a controlled experiment in diabetes means and involves.[3]

Above all, Lawrence was unequivocal about the relative merits of data versus ideas in advancing scientific knowledge—if the experimental data do not fit the preconceived idea, then we must abandon the idea, not the data.

> It is too easy to act like a lawyer pleading and proving his case and not like a factual scientist

Abbreviations: CaMKII calcium calmodulin dependent kinase II, DAG diacyl glycerol, Gi, Gs GTP binding proteins — inhibitory or stimulatory of adenylate cyclase, Gq G protein stimulating phospholipase C, GIP glucose dependent insulinotropic polypeptide, MAPK mitogen activated protein kinase, PACAP pituitary adenylate cyclase activating polypeptide, PKA cyclic AMP dependent protein kinase A, PKC protein kinase C, PLC phospholipase C, IP_3 inositol trisphosphate, VDCC voltage dependent Ca^{2+} channels.

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who must be ready to prove himself disappointingly wrong.[4]

Lawrence was a fine example of a clinician who cared deeply about scientific research because he realized the benefits that research could bring to his clinical practice. Nowadays, with increasing specialization in medicine and in science, there is often perceived to exist a division between clinical scientists and basic scientists, in which the former are medically qualified, motivated by a desire to help patients, and undertake research of direct relevance to the prevention or treatment of disease; while the latter are scientifically qualified, motivated by an unbridled curiosity, and undertake research in restricted and often esoteric areas of no apparent relevance to doctors or patients. This division is more imagined than real, and most basic biomedical scientists, like myself, work in our chosen areas precisely because we wish to generate knowledge which may be of therapeutic use to our clinical colleagues. In this short review I will attempt to explain some of the work which we have carried out at King's College London over the past decade in an area which may appear to be of little immediate interest to diabetologists and diabetic people, but which we believe is essential for the rational design of novel therapeutic strategies for the future.

Why Study β-Cells?

A quick search of any publications database shows a remarkable increase in the number of publications focused on pancreatic β-cells and islets of Langerhans over the past 15 years. In part, this may reflect the general trend towards more profuse publishing, driven by research assessment exercises, but it also reflects a growing consensus opinion amongst scientists that fully to understand and correct a pathological condition we must first understand the physiology of the normally functioning system. Understanding β-cell function therefore has two distinct goals, both of which have clinical and commercial potential.

First, identifying the molecular defects in Type 2 diabetes may enable novel therapies to be targeted to the site of the defects. The recent identification of the defects underlying some types of Maturity-Onset Diabetes of the Young (MODY) is an illuminating example. It was no great surprise to β-cell physiologists that mutations in the glucokinase gene are responsible for some cases of MODY,[5] but no one would have guessed that MODY could be attributed to mutations in genes coding for a number of transcription factors including HNF1α, HNF4α, and IPF-1.[6] In the absence of any information about the functions of these proteins in normal β-cells, it is difficult to determine why their dysfunction produces the MODY phenotype and the race is now on.

Secondly, while the transplantation of organ donor-derived β-cells, islets or pancreas offers the potential for curing Type 1 diabetes, the logistics of supply and demand of donor tissue suggest that this approach will make little impact on the clinical problem. There is therefore considerable current interest in the possibility of engineering replacement cells for transplants, either by equipping plentiful non-β-cells with the means of making and secreting insulin, or by manipulating the growth *in vitro* of authentic β-cells to provide unlimited transplant material.[7] Both approaches are completely dependent upon a detailed understanding of how normal β-cells recognize and respond to external stimuli with appropriate secretory or proliferative responses.

How Do β-Cells Recognize Signals?

Our understanding of β-cell physiology was greatly assisted by two major discoveries in the last two decades, both primarily driven by research in British laboratories. First, it became widely accepted that β-cells recognize nutrient stimuli by metabolizing them,[8] rather than through conventional receptors. This concept was developed to encompass 'initiators' and 'potentiators' of insulin secretion in which only nutrients are capable of initiating secretory responses, but the magnitude of the response to nutrients is potentiated by receptor-operated non-nutrient stimuli such as hormones and neurotransmitters.[8] Secondly, some clever electrophysiology demonstrated that the link between nutrient metabolism and insulin secretion is a K^+ channel in the β-cell plasma membrane whose conductance is dramatically reduced by the ATP generated from glycolytic metabolism.[9] A picture therefore emerged of the remarkable mechanisms which β-cells use to detect changes in blood glucose. The key features of this are included in the diagrammatic representation of a β-cell (Figure 1). When blood glucose concentrations rise postprandially, high-capacity transporters in the β-cell plasma membrane ensure similar elevations occur inside β-cells. The glucose is rapidly phosphorylated by the high-specificity, low-affinity glucokinase expressed in β-cells and the glucose-6-phosphate enters glycolytic and oxidative metabolic pathways with the consequent generation of ATP. The increased ATP, or changes in the ATP/ADP ratio, within the β-cell promotes the closure of the ATP-regulated K^+ channel (K^+_{ATP}) in the plasma membrane, causing the β-cell to depolarize with the consequent opening of voltage-dependent Ca^{2+} channels (VDCC). The huge Ca^{2+} concentration gradient across the plasma membrane drives an influx of extracellular Ca^{2+} into the depolarized β-cell through the VDCC, and this triggers the exocytotic release of insulin into the circulation.

At around the same time as the discovery of the K^+_{ATP} channel, molecular details of the mechanisms through which β-cells recognize non-nutrient stimuli were also being elucidated, and it became apparent that at least two distinct signalling pathways were involved. These are also included in Figure 1. Both start with signal recognition via cell surface receptors which are linked to their intracellular effector systems through heterotrimeric

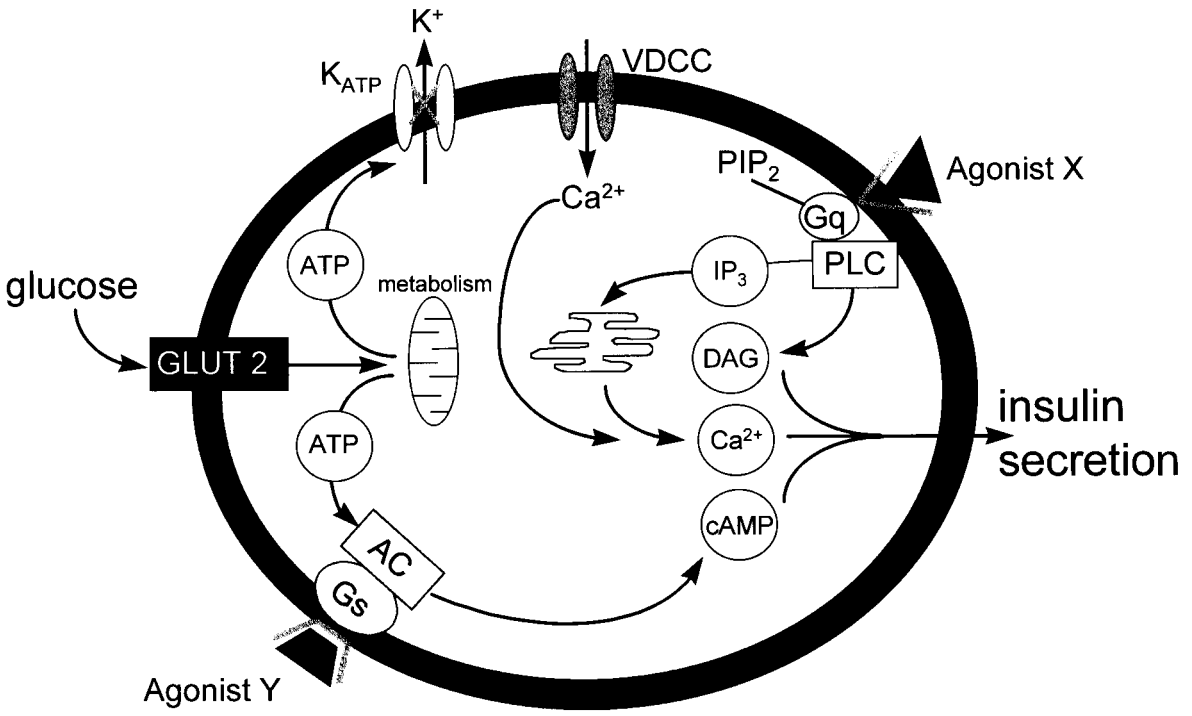

Figure 1. Signal recognition by pancreatic β-cells. The schematic diagram shows how β-cells transduce signals from nutrients (e.g. glucose) and non-nutrients (agonists X and Y). Glucose enters the β-cell on the GLUT2 transporter and is metabolized with a consequent generation of ATP and closure of K_{ATP} channels. The decreased efflux of K^+ leads to depolarization of the β-cell with the consequent opening of voltage dependent Ca^{2+} channels (VDCC) and an influx of extracellular Ca^{2+} down its concentration gradient. Nutrients may also activate phospholipase C (PLC) and adenylate cyclase (AC). Agonist X (e.g. acetylcholine) binds to cell-surface receptors which are coupled via the heterotrimeric GTP-binding protein G_q to PLC. Receptor occupancy activates PLC with the consequent generation of inositol trisphosphate (IP_3) and diacylglycerol (DAG) by the hydrolysis of membrane inositol phospholipids such as phosphatidyl inositol bis phosphate (PiP_2). IP_3 releases stored Ca^{2+} from the endoplasmic reticulum. Receptors for agonist Y (e.g. PACAP, GIP) are coupled to AC via G_s and receptor occupancy leads to the generation of cyclic AMP from ATP

GTP-binding proteins (G-proteins). Receptors associated with G_s or G_i stimulate or inhibit the activity of adenylate cyclase, respectively, so increasing or decreasing the production of cyclic AMP from ATP. Receptors associated with G_q stimulate the activity of phospholipase C (PLC), thus increasing the hydrolysis of membrane inositol phospholipids to produce diacylglycerol (DAG) and inositol phosphates such as inositol trisphosphate (IP_3). Thus, a whole range of different external signals can be translated into changes in the intracellular concentrations of a few regulatory molecules like Ca^{2+}, cyclic AMP, DAG, and IP_3.

How Do β-Cells Respond to Signals?

The detailed understanding of signal recognition mechanisms was a major advance in β-cell physiology, but it was only part of the story. Having recognized a signal, the β-cell must then mount an appropriate insulin secretory response and it is on this area that I wish to concentrate for the remainder of this article.

The key to understanding how signal recognition is transduced into secretory responses is the realization that the physiological stimuli for insulin secretion share the ability to increase the availability of Ca^{2+}, cyclic AMP or DAG and other products of phospholipid hydrolysis. What these intracellular regulators have in common is the ability to activate distinct classes of a type of enzyme known as protein serine/threonine kinases. These enzymes catalyse the transfer of phosphate from ATP to a serine or threonine residue in their specific protein substrates, and this alters the function of the substrate protein. For example, if the substrate protein is an enzyme, the phosphorylation may enhance or inhibit its catalytic activity. The phosphorylation process is reversible by another class of enzymes known as phosphoprotein phosphatases, so phosphorylation offers a selective, reversible means of regulating cellular function at the protein level.

By applying a wide range of different experimental approaches we, and other research groups around the world, have produced evidence that the activation of protein kinases is a key transduction step in regulating insulin secretion. A detailed consideration of the extensive literature is beyond the scope of this article, and aficionados are referred to our recent exhaustive review of this area.[10] However, it is worth considering briefly the experimental evidence suggesting an important role for protein phosphorylation in the regulation of β-cell

function, if only to gain an impression of the complexity of the transduction pathways involved.

Ca^{2+}-dependent Protein Kinases

As described above, the interaction between metabolic and ionic processes ensures that nutrients depolarize β-cells, allowing Ca^{2+} to enter the cell through voltage-dependent Ca^{2+} channels. The binding of cholinergic agonists to muscarinic receptors can also induce elevations in β-cell Ca^{2+} through the generation of IP_3, which liberates Ca^{2+} from intracellular stores rather than promoting an influx of extracellular Ca^{2+}. Secretagogue-induced elevations in β-cell Ca^{2+} can be mimicked in permeabilized β-cells, and such experiments have demonstrated that increased Ca^{2+} is alone sufficient to initiate a secretory response.[11,12] One target for intracellular Ca^{2+} is the ubiquitous protein kinase known as Ca^{2+}/calmodulin-dependent protein kinase II (CaMK II), as shown schematically in Figure 2. Activated CaMK II phosphorylates endogenous β-cell proteins,[12] initiating the insulin secretory response, and pharmacological inhibitors of CaMK II prevent protein phosphorylation and thus inhibit insulin secretory responses to physiological stimuli.[13] The activation of CaMK II therefore offers one mechanism through which Ca^{2+}-mobilizing stimuli can initiate insulin secretion.

Phospholipid-dependent Protein Kinases

Numerous isoforms of the Ca^{2+}/phospholipid-dependent protein kinase C family are expressed in β-cells, and there is convincing evidence that some of these are involved in responses to non-nutrient secretagogues. Agonists, such as acetylcholine or cholecystokinin, which activate receptors coupled to PLC stimulate the generation of both DAG and IP_3 within β-cells, as shown in Figure 2. These conditions favour the activation of some or all of the DAG-sensitive isoforms of PKC in β-cells (α, β, δ, ε), leading to increased phosphorylation of PKC substrates and enhanced insulin secretion.[14,15] The pharmacological activation of the DAG-sensitive PKC isoforms produces a profound and prolonged secretory response,[16] and experimental reductions in the expression of these PKC isoforms are accompanied by a loss of secretory responsiveness to agonists which act through PLC-linked receptors.[14,15] It is still unclear whether PKC activation plays any major role in secretory responses to nutrients,[17] and this has been the subject of considerable debate (see Jones and Persaud;[10] Persaud *et al.*;[17] Zawalwich and Rasmussen;[18] Wollheim and Regazzi[19]). On the one hand, nutrients are reported to activate some PKC isoforms in β-cells and many pharmacological inhibitors of PKC inhibit glucose-induced insulin secretion; but on the other hand, some inhibitors are reported to inhibit

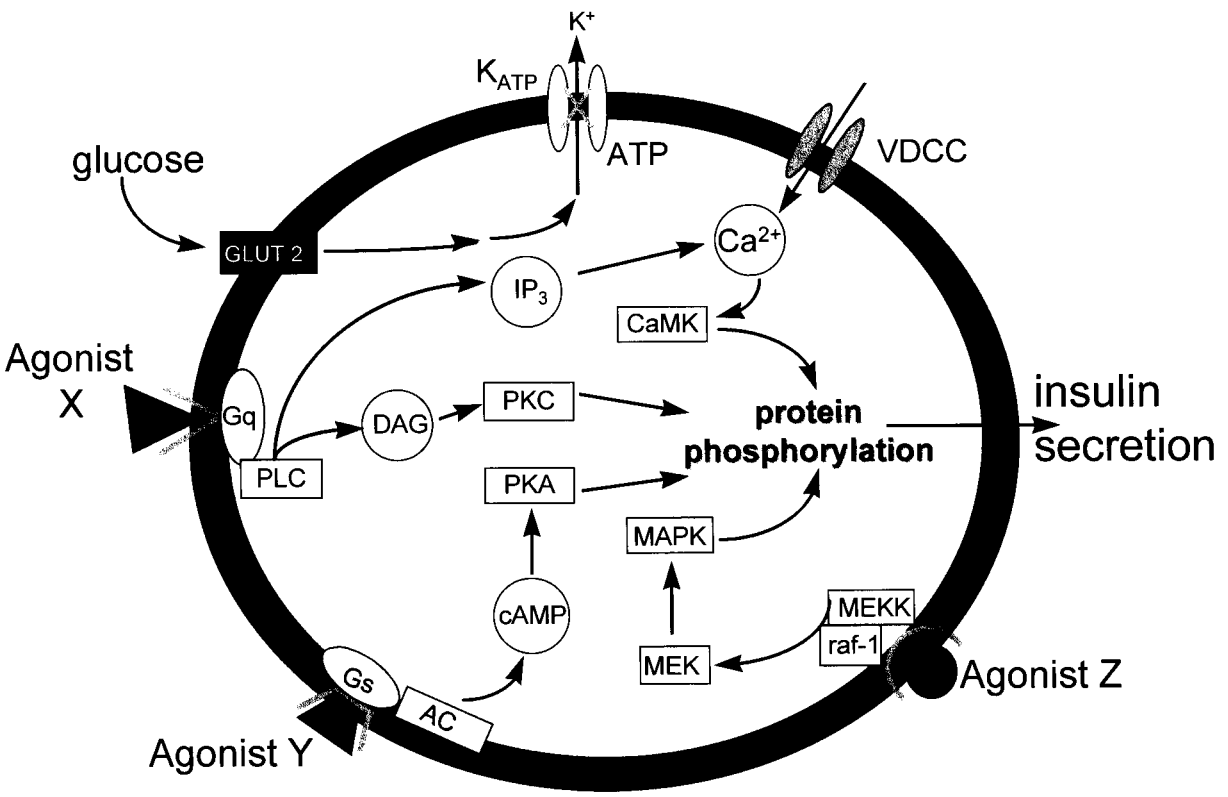

Figure 2. Protein kinases and the regulation of insulin secretion. The influx of extracellular Ca^{2+} caused by nutrient secretagogues activates the Ca^{2+}/calmodulin-dependent CaMK II. Agonist X (e.g. acetylcholine) may activate CaMK by the IP_3-induced release of Ca^{2+} from intracellular stores, and also activate PKC by the generation of DAG. Agonist Y (e.g. PACAP, GIP) activates PKA by increasing intracellular concentrations of cyclic AMP. Agonist Z (e.g. growth factors) may influence insulin secretion or β-cell proliferation through a cascade of protein kinases which results in the activation of MAP kinases (MAPK)

PKC activity without affecting responses to nutrients, and β-cells which are deficient in DAG-sensitive isoforms of PKC respond perfectly well to glucose and other nutrients. That debate is not yet over, but we can state with some confidence that PKC activation offers a transduction pathway through which hormones and neurotransmitters can potentiate insulin secretory responses which have been initiated by nutrients.

Cyclic AMP-dependent Protein Kinase

In many ways, the experimental observations underpinning a role for the cyclic AMP-dependent protein kinase (PKA) in insulin secretion resemble those for PKC. Thus, some receptor-mediated secretagogues, such as pituitary adenylate cyclase activating polypeptide (PACAP) and glucose-dependent insulinotropic polypeptide (GIP) act through G_s-coupled receptors to activate adenylate cyclase within β-cells, increasing intracellular cyclic AMP and activating PKA, as shown in Figure 2. Pharmacological activators of PKA stimulate the phosphorylation of β-cell proteins and are powerful potentiators of insulin secretion,[12,16,20,21] and pharmacological inhibitors of PKA inhibit cyclic AMP-induced insulin secretion.[21,22] Results like these suggest that the PKA pathway is used by non-nutrient secretagogues to potentiate secretory responses to nutrients in a manner similar to the PKC pathway. Although the involvement of PKA in responses to receptor-operated agonists is not disputed, there is considerable uncertainty about the involvement of PKA in nutrient-induced insulin secretion. As often happens in β-cell research, opinion is completely polarized between an obligatory role and a non-essential role for PKA in nutrient-induced insulin secretion. Thus, the activation of PKA has been suggested to be obligatory for the ability of β-cells to respond to nutrients, while other studies have demonstrated that inhibition of PKA had little or no effect on nutrient-induced insulin secretion (see Jones and Persaud[10]). In common with PKC, the debate may not be over, but we can again state with some confidence that the activation of PKA offers a transduction pathway through which non-nutrients can potentiate insulin secretory responses initiated by nutrients.

Other Protein Kinases

CaMK II, PKC, and PKA are attractive to β-cell physiologists because their activation can be precisely and specifically regulated by physiologically relevant external stimuli (summarized in Figure 2), but pancreatic β-cells also express many other protein kinases (including another class of enzymes which phosphorylate their substrate proteins on tyrosine residues), and many of these may prove to play important regulatory roles in β-cells. Although a full discussion is beyond the remit of this article, it is perhaps worth mentioning the mitogen-activated protein kinases (MAPK), which appear to be ubiquitous in mammalian cells, and include p42/44 MAP kinases, p38 reactivating kinase, and stress-activated protein kinases.

MAP kinases are activated by being phosphorylated on threonine and tyrosine residues by another kinase known as MAP kinase kinase (also known as MEK) which is, in turn, activated by the upstream kinases, MEK kinase and Raf-1. The components of this MAP kinase cascade are expressed in β-cells,[23] although their signalling function(s) are still unclear. It is possible that MAP kinases transduce the effects of growth factors on insulin secretion, as shown in Figure 2, although current evidence suggests that MAP kinase activation is neither sufficient nor essential for regulated insulin secretion.[23,24] Perhaps more importantly, MAP kinases are known to be involved in proliferative responses, and this family of enzymes may therefore provide a future target for the experimental manipulation of β-cell proliferation, which would have obvious and important therapeutic implications.

Why Do β-Cells Form Islets?

In deference to the reductionist approach of modern cell biology, during the course of this article and in the schematic diagrams, I have referred to the *pancreatic β-cell* as though β-cells exist as individuals whose function can be fully understood within the context of an individual cell. But β-cells do not exist alone *in vivo*, and the endocrine unit of the islet of Langerhans is a heterogenous collection of cells with a defined and complex anatomy. The question therefore arises as to why β-cells form these complex structures. The mechanistic answer is that β-cells express on their external surfaces a variety of cell adhesion molecules, some of which direct β-cell:β-cell interactions, while others ensure that the non-β-endocrine cells form a mantle around the β-cell core of the islet of Langerhans. However, the functional answer to the question is more interesting, since β-cells probably form islets because only by so doing can they produce the appropriate secretory responses to physiological stimuli. Experiments using dispersed islet cells and populations of purified β-cells have demonstrated that the integrated secretory response of β-cells within an islet is considerably greater than the sum of the responses of the individual β-cells in isolation,[25–27] although the reasons for this remain unclear.

The physiology of the isolated β-cell differs from that of β-cells in islets of Langerhans in several key aspects. For example, islets contain several endocrine cell types other than β-cells, and there is considerable scope for paracrine influences on β-cell function. However, paracrine effects alone cannot account for the improved secretory performance of islets over β-cells since glucose-induced insulin secretion is also improved in re-aggregates comprised of purified islet β-cells alone.[26,27] Perhaps more importantly, β-cells within islets are extensively

coupled through gap junctions which permit the passage of ions and small molecules between coupled cells.[28] Gap-junctional coupling within islets is not static, but changes with the secretory state of the islets,[29] and loss of gap-junctional communication is associated with impaired secretory responses to nutrients.[30,31] These observations suggest that communication between β-cells is essential for normal secretory responses to physiologically-relevant stimuli, and we are currently studying the functional consequences of inducing insulin-secreting cells to form islet-like structures *in vitro*.[32,33] This is another example of basic β-cell research which may have important clinical ramifications: current efforts to generate artificial β-cells for transplant therapy[7] may be of little value if the cells do not communicate with each other to produce the integrated responses of authentic islets of Langerhans.

Well, Would R.D. Lawrence Have Been Interested in the Regulation of Insulin Secretion from Pancreatic β-Cells?

This brief and biased overview of pancreatic β-cell physiology is an attempt to put into context some of today's basic science which may be of use to tomorrow's clinical practice. It is rarely possible to predict which avenues of basic research will prove useful; indeed, the entire value (and much of the fun) of basic research depends upon not knowing in advance what the results will be, or where a chosen line of research will lead. Throughout his career R.D. Lawrence was a staunch and vocal advocate of the potential benefits of research and I am sure that he would have been as interested in β-cell physiology as he was in any other research endeavour which might shed light on the pathogenesis or treatment of diabetes. Here is what he wrote about the value of research towards the end of his distinguished career, conveying a sentiment similar to that in the quotation which starts this article, although the two are separated by 40 years.

> I should like to stress the continuous need for Research…leading to a more complete understanding of the nature of diabetes and thence for a cure for the disease.[34]

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